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Published: 01/01/2015

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Citation for published version (APA):

Quinn, B., McGann, O., & Foley, V. (2015). *Environmental diagnostics: the use of medical diagnostic techniques to assess the health of the marine environment*. Paper presented at 18th International Symposium on Pollutant Responses in Marine Organisms, Trondheim, Norway.

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ENVIRONMENTAL DIAGNOSTICS: THE USE OF MEDICAL DIAGNOSTIC TECHNIQUES TO ASSESS THE HEALTH OF THE MARINE ENVIRONMENT

PRIMO Trondheim, 2015

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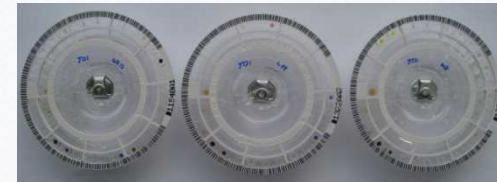
Aims of study

1. Investigate the potential of medical diagnostic technologies for use in environmental monitoring to assess the health status of animals (focus on *Mytilus spp.*)
 - Clinical chemistry
 - Immunoassay
2. Assess Impact of a contaminant on aquatic animals
 - Chronic effects of diclofenac on *Mytilus spp.* & rainbow trout
 - The effect of 17 α -ethynylestradiol (EE2) on steroid levels in *Mytilus spp.*

Abaxis Piccolo xpress™ clinical chemistry analyser

‘The Piccolo xpress™ is a compact, portable, fully automated Point of Care (POC) clinical chemistry analyser’

- Developed for human & veterinary samples
- Fully automated, simple to use
- Complete clinical chemistry analysis
- Test response of liver function tests
- Fast, 12 min for 13 endpoints
- Internal quality control system
- Using validated techniques



Mussel Hemolymph

- Clinical chemistry endpoints normally measured in blood
- Mussel hemolymph very dilute
- Need to develop protocol for concentration of hemolymph for use in diagnostic testing
- Hemolymph advantages: ease of sampling, little sample prep, sample over time, ethically acceptable

Piccolo xpress™ endpoints investigated

- **Rotor General Chemistry 13**

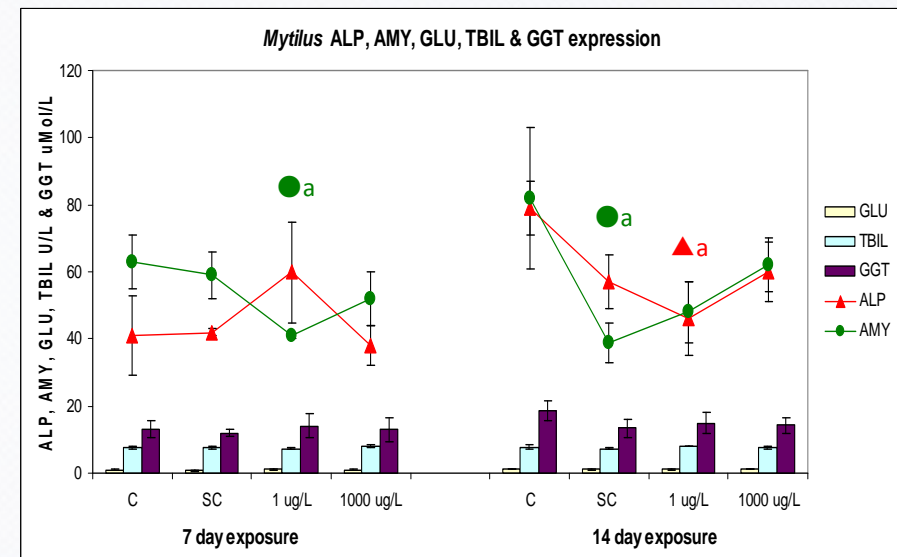
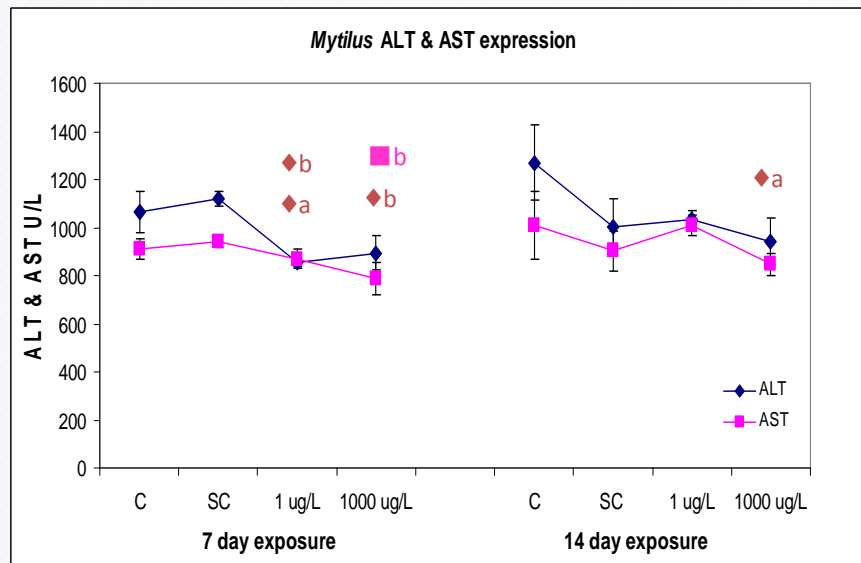
- Alanine aminotransferase (ALT) – Liver function test (inflammation)
- Aspartate aminotransferase (AST) – Acute liver damage
- Alkaline phosphatase (ALP) – Liver and bile duct
- Gamma-glutamyltransferase (GGT) – Liver function
- Total Bilirubin (TBIL) – Liver disorders
- Amylase (AMY) – Inflammation of pancreas
- Creatinine (CRE) – Renal disease
- Glucose (GLU) – Metabolism

Exposure of *Mytilus spp.* to diclofenac

- Semi-static (water change every 24h)
- C, SC (DMSO), 1 µg/L & 1000 µg/L
- Tanks in Triplicate
- 14 d exposure
- Sampled after 24h, 96h, 7d & 14d
- Samples taken for
 - Chemical analysis*
 - 2D GE analysis*
 - 'Traditional biomarkers'
 - Diagnostic endpoints



Clinical chemistry analysis of *Mytilus spp.* digestive gland exposed to diclofenac

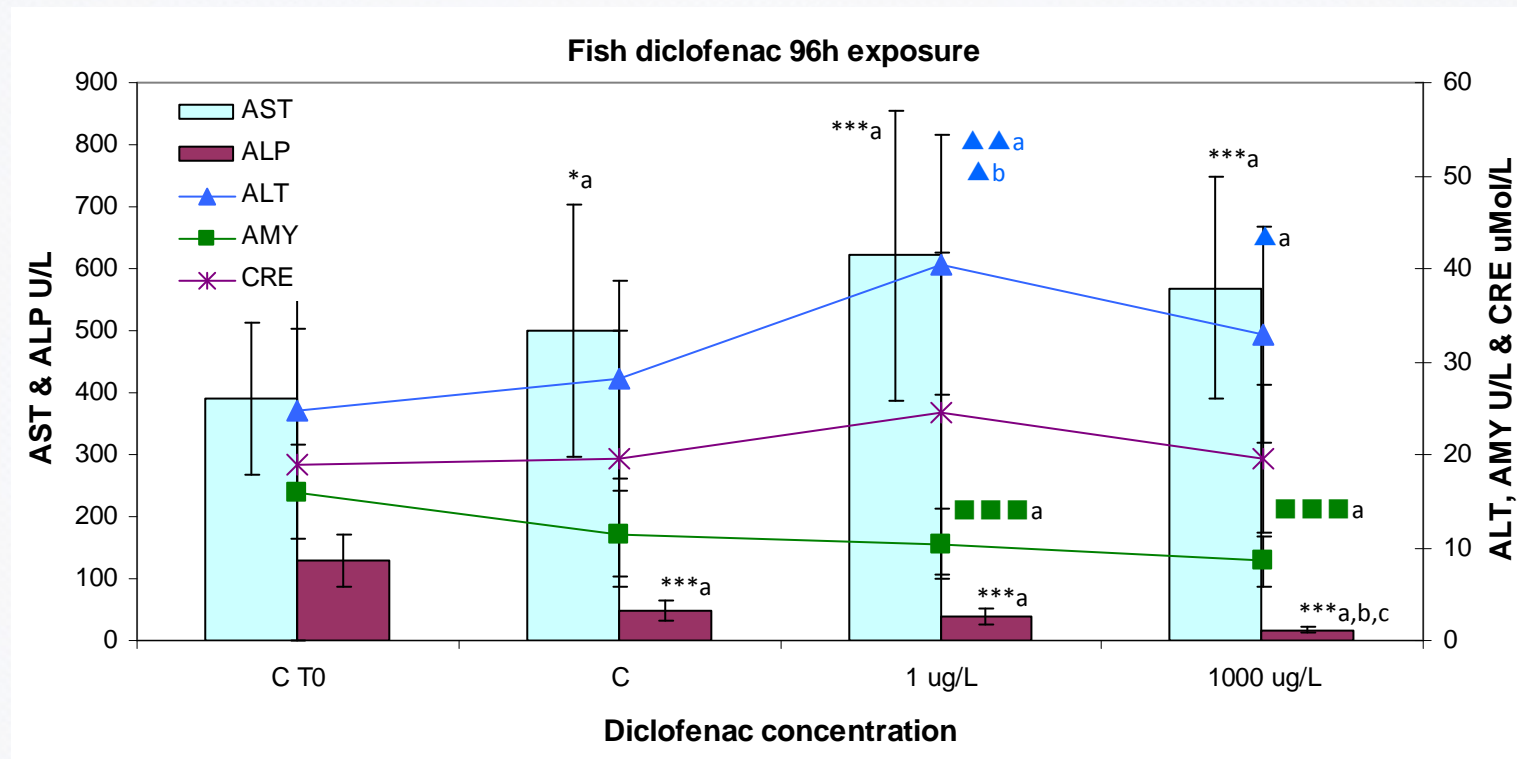


Exposure of Rainbow trout (*Oncorhynchus mykiss*) to diclofenac

- OECD guidelines 203 (fish acute toxicity test)
- Semi-static (water change every 24h)
- C, 1 $\mu\text{g/L}$ & 1000 $\mu\text{g/L}$
- Tanks in Triplicate
- 96h exposure
- Blood sampled after 96h
- Centrifuged 2000g, 10 min
- Serum analysed using GC13 rotor



Clinical chemistry analysis of Rainbow trout serum exposed to diclofenac



Siemens Immulite 2000 Immunoassay analyser

Solid phase competitive chemiluminescent enzyme immunoassay system

- Developed for human samples
- Semi-automated, simple to use
- Throughput of up to 200 tests/hour
- Comprehensive menu > 100 assays
- Internal quality control system
- Using validated techniques
- Potential for environmental monitoring?



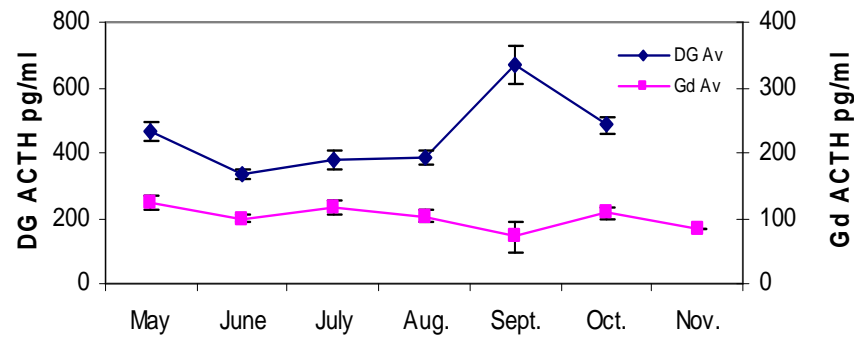
Steroid levels in *Mytilus spp.*

- Mussels sampled monthly over 12 month period
- Presenting results for May – Oct/Nov
- Mussel Digestive gland (DG) and gonad (Gd) dissected
- Homogenised in ice cold buffer (130 mM NaCl, 25 mM Hepes- NaOH containing 1 mM EDTA & 1 mM dithiothreitol, pH 7.4, at 4°C)
- Centrifuged at 15,000 rpm for 60 min @ 4°C
- S15 frozen at -80°C until analysis
- Samples defrosted & immediately run on Immulite

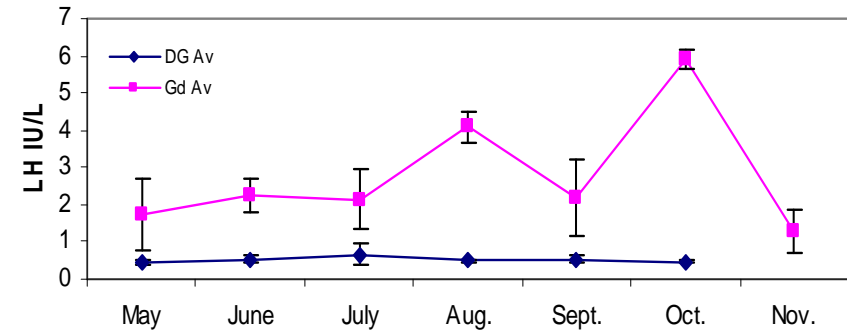
Endpoints

1. **Adrenocorticotrophic hormone (ACTH)**; Increases production & release of corticosteroids, ultimately results in steroidogenesis
2. **Estrogen**; Primary female sex hormone. Regulates functions of the reproductive system
3. **FSH Follicle-stimulating hormone (FSH)**; Regulates the development, growth, maturation & reproductive processes of the body.
4. **Luteinizing hormone (LH)**; In females triggers ovulation. In males stimulates production of testosterone. FSH & LH act synergistically
5. **Testosterone (TES)**; Androgen steroid hormone. Principal male sex hormone. Primarily secreted in the testicles and ovaries
6. **Progesterone (PROG)**; Involved in the female menstrual cycle, pregnancy & embryogenesis. Produced in the ovaries

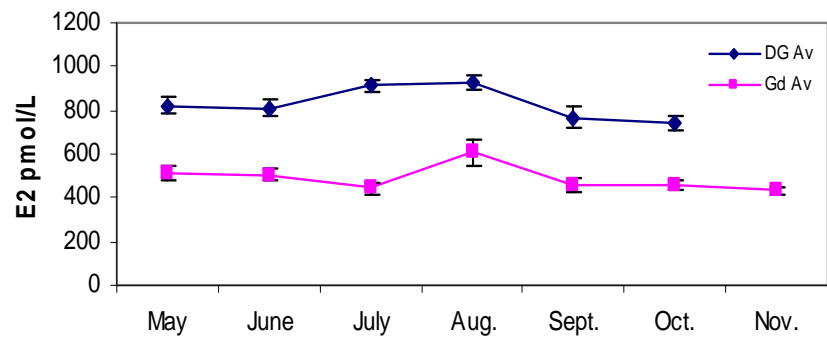
Adrenocorticotrophic hormone



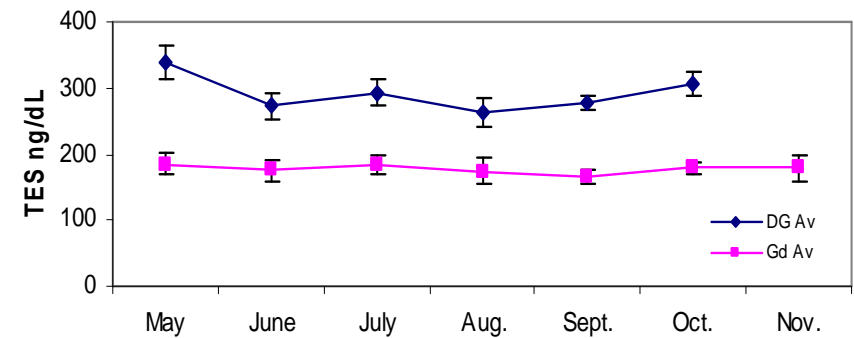
Luteinizing hormone



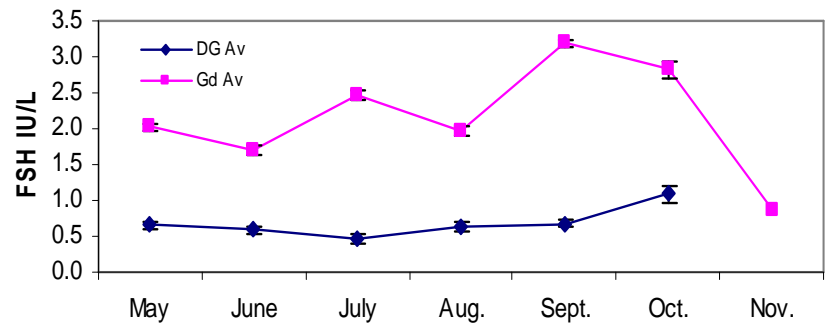
Estrogen



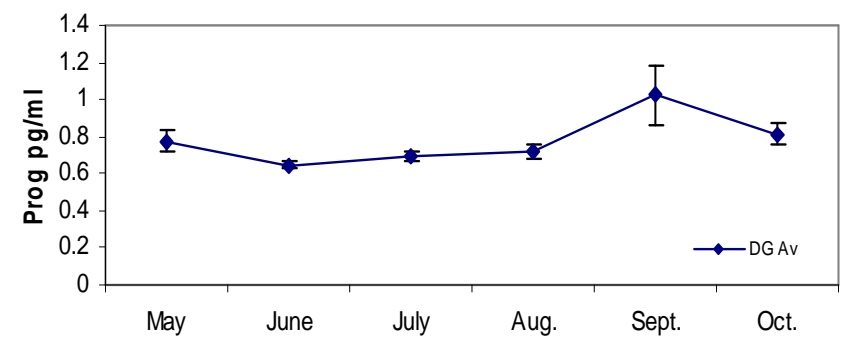
Testosterone



Follicle-stimulating hormone



Progesterone

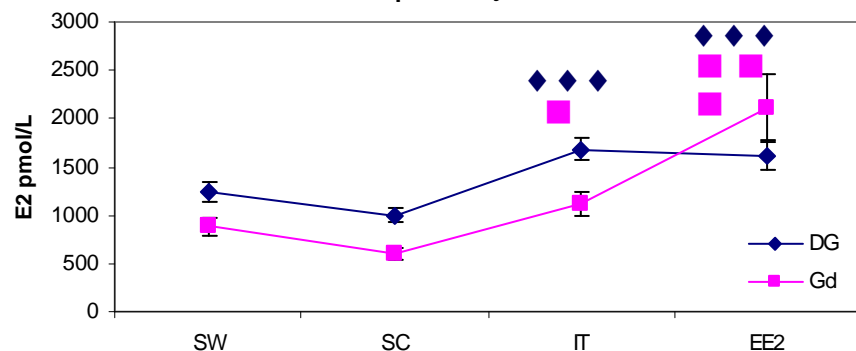


Mytilus spp. 17 α -ethinylestradiol exposure

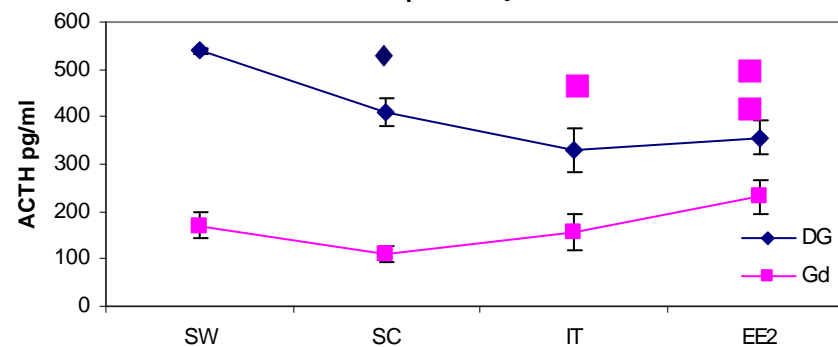
- *Mytilus spp.* taken from reference site (5-6 cm)
- Semi-static exposed to EE2 for 7 day using artificial seawater
- 60 mussels in 60 L tanks
- Concentration of 150 ng/L EE2
- Solvent (ethanol) concentration (0.000015%)
- Exposures:
 - Seawater Control (SW)
 - Solvent Control (SC)
 - Intertidal EE2 exposed (IT)
 - Submerged EE2 exposed (EE2)

Steroid levels in *Mytilus spp.*: Findings

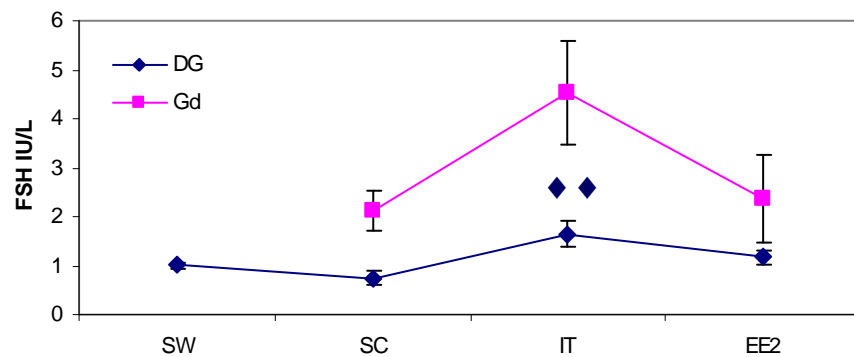
E2 EE2 exposed *Mytilus*



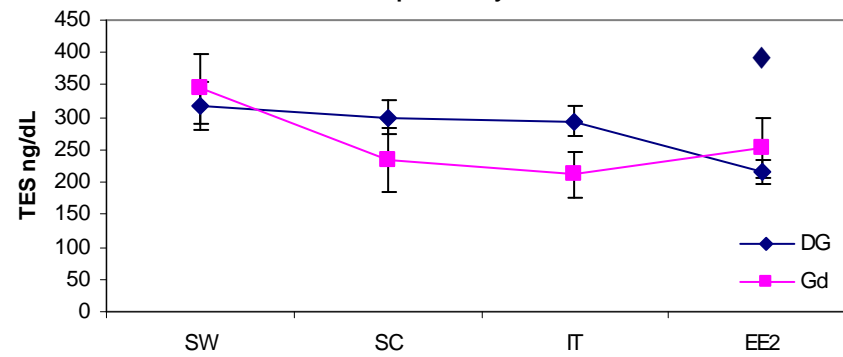
ACTH EE2 exposed *Mytilus*



FSH EE2 exposed *Mytilus*



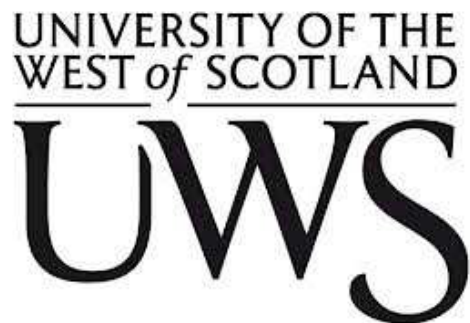
TES EE2 exposed *Mytilus*



Conclusion

1. Clinical chemistry endpoints measurable and impacted following diclofenac exposure in *Mytilus spp.*
2. Can measure steroid levels in *Mytilus* over time and the impact of EE2 exposure with human based immunoassays
3. Opportunity for direct inter-species and inter-phyla comparison – ecosystem level approach
4. More validation needed, but initial results indicate these diagnostic technologies suitable for environmental monitoring

Acknowledgements:

The Siemens logo, featuring the word "SIEMENS" in a bold, teal, sans-serif font.The Cruinn logo, featuring the word "Cruinn" in a stylized, green, serif font, with "Cruinn Diagnostics Limited" in a smaller, black, sans-serif font below it.The University of the West of Scotland (UWS) logo, featuring the text "UNIVERSITY OF THE WEST of SCOTLAND" in a small, black, sans-serif font above the large, bold, black, serif letters "UWS".The Environmental Protection Agency (epa) logo, featuring a stylized green leaf icon above the lowercase letters "epa" in a blue, sans-serif font, with "Environmental Protection Agency" in a smaller, blue, sans-serif font below it.

Thank you for your attention...

Questions?

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The University of the West of Scotland (UWS) logo, featuring the text "UNIVERSITY OF THE WEST of SCOTLAND" in a small, black, sans-serif font above the large, bold, black, serif letters "UWS".